INHIBITION OF CYTOTOXICITY OF GOAT ANTIBODIES AGAINST MOUSE THYMOCYTES BY MOUSE SERUM

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UDC 616-006-092:612.017.1]-07

KEY WORDS: thymocytes; cytotoxicity; target cells.

Sufficient evidence has now been obtained to show that tumor development in animals and man is accompanied by production of antibodies (AB) [5, 7, 8]. It has also been shown that these AB have a protective action on tumor cells and may even accelerate tumor growth [2, 4, 5]. The most interesting fact is that AB, protecting tumor growth, are complement-fixing and, consequently, cytotoxic. In the case of tumor growth, however, they have no cytotoxic action. Different hypotheses have been put forward to explain this absence of cytotoxicity of AB by a deficiency of complement or a deficient quantity of antibodies compared with the number of target cells. The corresponding hypotheses have been verified experimentally [1]. Nevertheless, it is very difficult to understand why during tumor growth relationships should arise between AB and complement or AB and target cells that are not sufficient for them to exhibit cytotoxicity in vivo. From our point of view it is more logical to postulate that conditions blocking the cytotoxicity of AB, both against tumor cells and against the host's tissues, exist in the body of a tumor-bearing, just as in a healthy individual.

To test this hypothesis we chose AB known to be cytotoxic, and ratios of AB to complement and to target cells necessary for exhibition of their cytotoxicity, and we studied the effect of syngeneic (relative to target cells) animal serum and its components on the cytotoxicity of AB under effective conditions.

EXPERIMENTAL METHOD

Unvaccinated male C57BL/6, Balb/c, and (C57BL/6/CBA) hybrid mice aged 3 months, were obtained from the Stolbovaya nursery, Academy of Medical Sciences of the USSR. The animals were killed by cervical dislocation, and their blood was collected in centrifuge tubes to obtain serum. The thymus was dissected, and thymus cells were flushed out by repeated centrifugation at 800*g* for 5 min, twice in medium 199. The globulin fraction (GF) of the mouse blood serum was obtained by precipitation of proteins with ammonium sulfate. The protein residue was dissolved in a volume equal to that of the original blood serum, and the ammonium sulfate was removed by dialysis. The low-molecular-weight fraction (LF) was obtained from the supernatant of the serum after precipitation of GF by ammonium sulfate, which was dialyzed, freeze-dried, and dissolved in a volume equal to that of the original serum. The substances were dialyzed and dissolved in 0.15 M NaCl, 0.02 M phosphate buffer, pH 7.4.

Antibodies. Cytotoxic goat AB against mouse thymocytes were obtained from "Calbiochem."

Complement. Fresh human blood serum was used.

Conduct of the Tests. 50 μl of a suspension of thymocytes, with 5 million cells in 1 ml of medium 199, 50 μl of factor or medium 199, 50 μl of cytotoxic AB in medium 199, and 50 μl of active or inactive complement were used. Cytotoxicity was recorded by staining the cells with trypan blue or measuring release of ^{51}Cr. Dead cells were counted in a hemocytometer under the microscope or by means of a "Beckman" DF-5500 γ-counter. The results were subjected to statistical analysis by the Fisher–Student test. Differences were considered to be significant at the $p < 0.05$ level. Each experiment was repeated at least 3 times.

Oncologic Scientific Center, Russian Academy of Medical Sciences (Presented by Academician of the Russian Academy of Medical Sciences N. N. Trapeznikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 6, pp. 642-644, June, 1992. Original article submitted November 12, 1991.

TABLE 1. Effect of Complement on Cytotoxicity of Antithymocytic AB (% of dying cells is shown)

Series No.	Groups	Titer of complement							
		1	2	4	8	16	28	32	64
I	TC + C	5	5	2	5	3	5	5	2
II	TC + AS + C	99	97	96	90	51	47	39	40
III	TC + AS + C + MS	5	2	5	4	5	2	3	2
IV	TC + AS + Ct^0 + MS	3	4	3	3	3	3	3	3

Legend. TC) Thymocytes, C) complement, AS) antithymocytic goat serum, Ct°) complement inactivated by heat, MS) mouse serum. Mean results of three measurements are given. Differences between II and I, II and III, and II and IV groups significant, $p < 0.001$.

TABLE 2. Effect of Syngeneic Serum and LF on Cytotoxicity of Antithymocytic AB (% of dying cells is shown)

Series No.	Groups	Titer of antiserum		
		5	25	50
I	TC + AS + C	99	98	93
II	TC + AS + Ct^0	2	2	2
III	TC + AS + C + MS	4	2	3
IV	TC + AS + Ct^0 + MS	2	2	2
V	TC + AS + C + LF	99	93	93
VI	TC + AS + Ct^0 + LF	2	2	2

Legend as to Table 1. Mean results of three measurements are given. Differences between groups I and II, I and III, and V and III are significant, $p < 0.001$.

EXPERIMENTAL RESULTS

Table 1 gives the results relating to the effect of different dilutions of complement on cytotoxicity of antithymocytic AB. It will be clear that in group I, in which thymocytes were incubated with original and diluted complement (titers 2, 4, 8, 16, 28, 32, 64), mortality of the cells was low (up to 6%), and independent of the titer of complement. The percentage of thymocytes killed on addition of the antiserum (group II) rose sharply with a high concentration of complement to 80% or higher, and with a titer of complement of under 16, mortality of the cells was below 50%. On the addition of syngeneic (relative to the thymocytes) mouse serum (group III) to this combination, which was cytotoxic for thymocytes, the observed cytotoxicity of the antithymocytic serum was completely abolished, and the percentage of dying cells in this group was independent of the titer of complement and did not exceed 5%, i.e., the mortality level of the cells in the control. A similar absence of cytotoxicity of the antiserum was observed when complement inactivated by heating to 56°C was used (group IV).

Table 2 gives results showing the effect of syngeneic plasma and LF on the cytotoxicity of antithymocytic serum. Initially the antiserum and complement had a strong cytotoxic action under the conditions studied on the thymocytes, causing death of more than 90% of cells with antiserum in a titer of 5, 25, and 50 (group I). Initially the antiserum itself did not possess cytotoxicity, for on inactivation of complement by heat, mortality of the thymocytes did not exceed 4% (group II). Meanwhile, addition of serum syngeneic relative to thymocytes to the cytotoxic system caused a distinct protective effect, and mortality of the cells in the presence of serum did not exceed 4% (group III). This protective effect was not connected with albumins or other molecules with mol. wt. of under 60 kD, for this

TABLE 3. Effect of Syngeneic Serum and Globulin Fraction on Cytotoxic Effect of Antithymocytic AB (% of dying cells is shown)

Series No.	Groups	Titer of antiserum		
		5	25	50
I	TC + AS + C	94	96	75
II	TC + AS + C + GF	42	46	27
III	TC + AS + MS	4	5	4
IV	TC + AS + C + GF	5	3	4
V	TC + AS + C + MS	11	14	3
VI	TC + AS	9	8	8

Legend. GF) Globulin fraction; rest of legend as to Table 1. Results of three measurements given. Differences between groups I and II and I and V are significant, $p < 0.05$.

TABLE 4. Effect of Syngeneic Serum on Cytotoxic Effect of Antithymocytic AB (% of dying cells is shown)

Series No.	Groups	Titer of complement				
		1	5	25	50	100
I	TC + AS + C + MS	21	81	85	79	80
II	TC + AS + C	88	86	92	92	91

Legend as to Table 1. Mean of three measurements shown. Differences between groups I and II for titer 1 are significant, $p < 0.05$.

blood serum fraction does not protect thymocytes against the cytotoxic action of AB, and the mortality of the cells in group V reached 99%. In groups II, IV, and VI, in which the cells were incubated with inactivated complement, aggregation of the cells under the influence of AB was observed. The fact of platelet aggregation in the presence of syngeneic serum suggests that the latter does not block interaction of cytotoxic AB with target cells.

It will be clear from Table 3 that the globulin fraction of syngeneic serum reduced the cytotoxicity of the antithymocytic AB by a factor of 2-3, lowering the percentage of dying cells from 96-75% (group I) to 46-27% (group II). The original syngeneic serum had a stronger protective action on thymocytes, and the percentage of dying cells did not exceed 14 (group V).

Similar results indicating abolition of the cytotoxic action of antithymocytic goat AB of mouse thymocytes in the presence of mouse serum were obtained also by recording death of the cells as reflected in release of the isotope ^{51}Cr (data not given).

It is important to note that diluting the syngeneic serum sharply reduced its ability to block the cytotoxicity of goat AB (Table 4). For instance, with undiluted serum, mortality of the cells averaged about 21%, compared with 88% in the control (groups I and II respectively). On dilution of the mouse serum fivefold or more, its ability to block the cytotoxicity of AB disappeared, and the percentage of dying cells was about 80-90% in both groups.

It can thus be concluded from these results that mouse serum and, in particular, its globulin fraction, contains factors capable of blocking the complement-dependent cytotoxic action of goat AB against mouse thymocytes. The mechanism of blocking of AB activity is not yet known. However, the presence of cell aggregates together with antithymocytic AB, and the fact that the experiments were carried out on unvaccinated animals of different strains and batches, suggest that blocking of the cytotoxicity of AB was unconnected with interaction between mouse immunoglobulins and goat AB as a result of preliminary sensitization of the mice with goat immunoglobulins. This phenomenon can therefore be logically explained by the existence of factors capable of blocking cytotoxic AB under

normal conditions in the serum. Continuation of the study of this phenomenon is indicated, for disturbance of equilibrium between AB and factors blocking their activity could lead to the development of autoimmune and neoplastic diseases.

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